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# 24
# Breeding for Grain Amino Acid Composition in Maize

Audrey Darrigues, Department of Agronomy, Iowa State University
Kendall R. Lamkey, Department of Agronomy, Iowa State University
M. Paul Scott, USDA-ARS, Corn Insects and Crop Genetics Research Unit

Improving the amino acid balance of grain has been a long-standing objective of plant-breeding research. In this chapter, we review the history of maize breeding for improved amino acid balance. Following this, we present results of our experiments involving divergent selection for the levels of the amino acids tryptophan and methionine in random-mated populations.

The majority of maize produced worldwide is used for food and feed, so one of the best ways to improve the value of this grain is to improve its nutritional quality. The main nutritional limitation of maize is that it is not a good source of protein. While maize grain typically contains 4–10% protein, this protein has less dietary value than protein from animal sources. This is because plant proteins tend to be digested less efficiently and are more likely to cause antigenic responses than animal proteins commonly used in diets. More importantly, plant proteins are deficient in certain amino acids, while other amino acids are in excess relative to the needs of animals (Figure 24.1). This imbalance in amino acid content decreases the nutritional value of plant proteins in animal diets.

## Essential amino acids

Monogastric animals (including humans) require specific dietary essential amino acids. By definition, amino acids are deemed essential if an organism does not synthesize them and they must therefore be supplied in the diet. If one of the essential amino acids is limiting, the deficiency results in a negative nitrogen balance (Berg, 2002). Nonessential amino acids are not required per se in the diet but are required for protein synthesis (Cheeke, 1999) and therefore must be either supplied in the diet or synthesized from dietary components. Therefore, a nonessential amino acid may be the limiting factor in growth if its level in the diet is insufficient and if the essential amino acids from which it is made are present in marginal amounts (Wiseman, 1987).

In animal diets, the efficiency of protein utilization is dependent upon two types of factors: external factors, which relate to rearing conditions, and internal factors, relating directly with the protein itself (Berg, 2002). The nutritional value associated with a protein may be estimated by comparing the ingested nitrogen to that which is actually retained for protein synthesis. Requirements for a specific monogastric animal depend on its metabolic peculiarities and are dependent on the genotype, performance, method of feeding, and the environment in which the animal is reared (Wiseman, 1987). Also, the protein quality requirement is different for growth than for maintenance and is affected by sex and by species (Cheeke, 1999). Signs of protein deficiency include anorexia, reduced growth rate, reduced or negative nitrogen balance and reduced efficiency of feed utilization. Specific lesions may appear with deficiencies for certain amino acids: tryptophan deficiency produces eye cataracts; methionine deficiency produces fatty liver (Pond, 1995). In humans, diets with imbalanced amino acid levels contribute to the malnutrition conditions of Kwashiorkor and Marasmus.

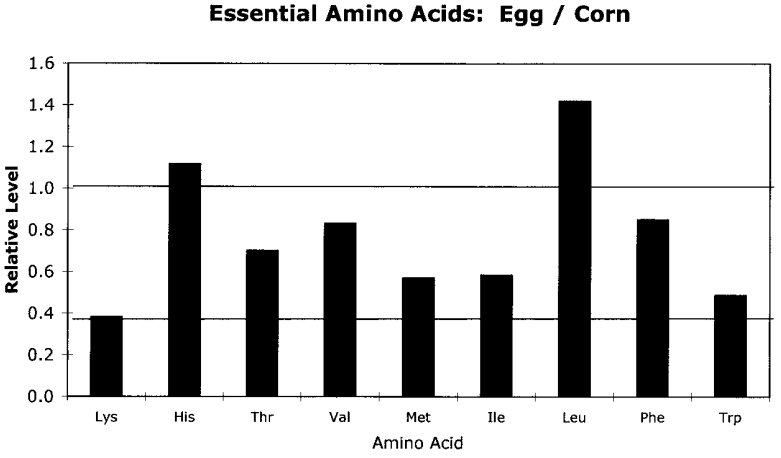


**Figure 24.1** For each amino acid, the ratio of the level in egg to the level in corn is presented. Egg is considered a nearly balanced source of protein, so this illustrates the deficiencies and surpluses of corn protein.

## Amino acid levels in maize

### *What determines amino acid levels*

Osborne (1924) classified seed proteins into four classes based on their solubilities. These classes are the albumins (water soluble), the globulins (salt soluble), the prolamins (aqueous alcohol soluble), and the glutelins (not soluble in water, saline solutions, or aqueous alcohol). This nomenclature is still used today. In most cereals the most abundant seed storage proteins are prolamins, while in dicots the most abundant seed storage proteins are usually globulins. In each genus, the major seed storage protein is named on the basis of the genus name, thus the major seed storage proteins in maize are called the zeins for the genus *Zea* and belong to the prolamin class of proteins. Similarly, the major seed storage proteins of soybean are globulins called glycinins after the genus *Glycine.*

The zeins can account for 40–60% of the total protein in the maize endosperm, and, because of their abundance, they are the primary determinants of the amino acid composition in maize kernels (Larkins et al., 1993). Osborne and Clapp (1908) characterized the amino acid composition of the zein proteins and reported that they lack two essential amino acids, lysine and tryptophan. This deficiency is reflected in the amino acid balance of maize, as illustrated in Figure 24.1. Therefore, increasing the levels of lysine and tryptophan are important goals for plant-breeding efforts directed to improving grain amino acid balance.

Maize-based feed is often supplemented with oil-seed by-products such as soy protein. These by-products complement the amino acid balance of maize protein somewhat, resulting in a more balanced diet. However, because the globulin storage proteins of dicots are deficient in the sulfur amino acids cysteine and methionine, increasing methionine levels is another important goal of plant-breeding programs.

### *Genes involved in determining amino acid balance*

Several mutant genes affect the amino acid balance in maize. Generally, these mutants alter the accumulation of zeins. An example is *opaque-2,* first described in 1935. Homozygous kernels carrying this mutation have a low density and do not transmit light because of their floury nature. Kernels with this phenotype have elevated levels of lysine (Mertz et al., 1964). This change is accompanied by a reduction in the levels of alpha zeins (Mertz et al., 1964), which are low in lysine content. The gene product of *Opaque-2* is a transcription factor involved in regulation of zein synthesis (Hartings et al, 1989; Schmidt, et al, 1990).

The *floury-2* mutant (Mumm, 1935) has a phenotype similar to *opaque-2* and has an altered amino acid balance with high concentrations of lysine and methionine (Nelson et al., 1965). In this mutation, a modified zein that cannot be processed properly interferes with accumulation of proteins dependent on the secretory system, including the zeins (Coleman and Larkins, 1995).

Thus, *opaque-2* and *floury-2* both achieve similar phenotypes with floury kernels and improved lysine content by similar processes. Both muta-

tions result in reduced zein deposition, although *opaque-2* contains a transcriptional defect and *floury-2* contains a translational defect. Because the zeins that are reduced in these mutations have very little lysine, the overall effect is an increase in lysine concentration.

A mutation that results in increased levels of kernel methionine has been characterized. This mutation results in overproduction of 10-kDa delta zein that is rich in methionine (Phillips and McClure, 1985). The overproduction of this zein is attributed to a regulatory gene *Zpr10/22* (Benner et al., 1989) that regulates delta zein accumulation posttranscriptionally (Cruz Alvarez et al., 1991). The *Zpr10/22* locus was later renamed *Dzr1* (Chaudhuri and Messing, 1994).

## Studies on quality traits in maize

### *Traditional breeding for quality traits*

#### Selection for protein quantity

A number of maize breeders have used traditional breeding methods to increase the level of protein in maize kernels. In 1896 Hopkins initiated a selection program for protein and oil content (Hopkins, 1899) that developed into the Illinois long-term selection experiment. This experiment consisted of repeated cycles of divergent selection for oil and protein and was highly successful in changing kernel composition in maize. After 70 cycles of selection, the protein content of the high-protein (IHP) and low-protein (ILP) strains was 26.6% and 4.4%, respectively (Dudley, 1974).

The major agronomic difference between the IHP and the ILP populations is grain yield. IHP plants yield substantially less than ILP plants. The chemical kernel composition of strains derived from the high and low selection for protein and oil has been studied extensively for physiological and biochemical modifications. Differences have been associated with the nitrogen (N) and carbon (C) metabolisms in the plant, which is influenced by the uptake, assimilation, translocation, and utilization of N and C.

IHP strains are more efficient at absorbing and translocating N (Lorenzoni et al., 1978; Wyss et al., 1991) and have a higher capability to assimilate nitrate in the roots (Lohaus et al., 1998), a higher capacity for amino acid transport to the grain (Reggiani et al., 1985; Lohaus et al., 1998), elevated asparagine levels (Lohaus et al., 1998), higher N-metabolism enzyme activity (Lohaus et al., 1998), and limited remobilization of leaf N (Wyss et al., 1991). In addition, the IHP strains have a higher level of seed phytic acid than ILP (Raboy et al., 1989), higher levels of amino acids and lower levels of sugars than ILP (Reggiani et al., 1985), higher enzyme activity (Reggiani et al., 1985), higher enzyme activity associated with starch accumulation (Lorenzoni et al., 1978), greater endoreplication, and higher ploidy level (Cavallini et al., 1995). ILP have lower levels of zein (Lorenzoni et al., 1978).

Frey et al. (1949) suggested that selecting for an increase in total protein in the maize kernel results in an increase in the zein fraction in the endosperm proteins. Similarly, Reggiani et al. (1985) concluded that in maize, long-term selection for diverging levels of protein in the grain has resulted in diverging levels of storage proteins in the endosperm. Dr. Fred Below of the University of Illinois suggests that it is primarily the accumulation of 19- and 22-kDa alpha zeins that have been altered by selection in the Illinois long-term selection experiment (personal communication). Given that the zein fraction has poor nutritional quality due to its lack of tryptophan and lysine, it seems likely that selection for protein content will result in lower protein quality. In 1951, Frey concluded from his study on the interrelationships of proteins and amino acids in corn that the protein in selections for low protein was more nutritionally balanced than the protein in selections for high protein. Thus, in order to improve protein quality by selecting for protein quantity, it would be best to select for low protein (Frey, 1951).

#### Selection for protein quality

Zuber and Helm (1972) studied the improvement of protein quality, defined as an increase in the lysine content of open-pollinated varieties, without the use of endosperm mutants. They used a recurrent selection method as a means of improving the amino acid balance. They were able to increase the level of lysine using two cycles of selection, though the mean protein values remained essentially the same. They also suggest that different environmental conditions could have caused such changes because the two cycles were grown in different seasons (Zuber and Helm, 1972).

### *Mutation breeding and QPM*

The findings of Mertz, Bates, and Nelson in 1964 that the *opaque-2* mutant has a lower content of the zein proteins in the endosperm and also provides 69% more lysine than wild-type maize kernels (Mertz et al., 1964) changed the emphasis of plant breeding for amino acid balance from recurrent selection to mutation breeding. Unfortunately, pleiotropic effects of the *opaque-2* phenotype complicated breeding efforts. These effects are reduced grain yield; soft and chalky kernel phenotype; greater vulnerability to ear rot; greater moisture content, which conflicted with the drydown of the seed; and lower rate of germination (Vasal, 2001). Efforts to improve the *opaque-2* phenotype were initiated at CIMMYT in the mid-1970s. The combination of two mutants, *sugary-2* and *opaque-2*, was found to be slightly better than *opaque-2* maize in terms of kernel hardness and ear rot tolerance, but grain yield and germination rate was not improved. However, the protein quality of the double-mutant combination was sometimes better than that of the *opaque-2* maize (Mertz, 1992).

In the 1980s, CIMMYT engaged in developing quality protein maize (QPM) by combining the *opaque-2* gene with genetic modifiers that improved the hardness of the maize kernel. Eventually, the scientists at CIMMYT were able to develop QPM material that yielded as well as their normal counterparts and contained the improved amino acid balance conditioned by the *opaque-2* mutation (Vasal, 2001).

### *Biotechnology approaches to improving protein quality*

With the advent of genetic engineering, a number of studies have proven the feasibility of improving the methionine content in a variety of crops. Lai and Messing (2002) constructed a transgene based on a chimeric *Dzs10* gene by replacing the 3′ UTR with a transcript of the cauliflower mosaic virus that would enhance the level of expression in maize endosperm cells. The level of methionine was increased as a result of the accumulation of the Dzs10 protein, a high-methionine zein. Because milk protein has the potential to provide good nutritional enhancement with its excellent amino acid profile, Yang et al. (2002) sought to synthesize a porcine α-lactalbumin gene construct. Expression of this synthetic gene in maize kernels resulted in a 20% increase in lysine levels (Bicar et al., unpublished). Transformation of narrow-leafed lupin (*Lupinus angustifolius* L.) seeds expressing the sunflower seed albumin (SSA) gene resulted in a 94% increase in methionine when compared with the wild-type (Molvig et al., 1997). Molvig et al. reported that not only was the protein quality improved in the transgenic seeds, but also the true protein digestibility, the biological value, and the net protein utilization. In tobacco, a group of researchers created and transformed a chimeric gene encoding a Brazil nut methionine-rich seed protein (Altenbach et al., 1989). The accumulation of the protein in the tobacco seeds resulted in a 30% increase in the levels of methionine.

Biotechnological approaches in improving the nutritional quality of crops may be promising, though both advantages and disadvantages have to be elucidated. Benefits of such technology are that genes expressing protein from a different organism than the target host can have beneficial nutritional attributes. In the study conducted by Yang et al. (2002), a porcine milk protein with good digestibility, bioavailability, and amino acid balance was introduced into maize. Disadvantages of using biotechnological tools are the difficulties associated with plant transformation and expression of foreign proteins and the potential introduction of allergenic properties. Several studies have reported such difficulties. Molvig et al. (1997) reported that molecular approaches in improving the amino acid balance were hindered by the difficult regeneration of grain legumes and by the unstable expression of the modified protein in the target host. The methionine-rich Brazil nut protein expressed in soybean may have had unfavorable allergenic properties (Nordlee et al., 1996).

## Methods for quantifying amino acids in maize kernels

In any plant-breeding program aimed at improving the content of amino acids, it is critical to have a method for quantifying the amino acids of interest accurately and inexpensively. Recent advances in automation, especially liquid-handling technology, greatly facilitate these measurements. Small-scale, replicated assays can be conducted efficiently in 96-well-plate format for a fraction of the cost of older analytical methods.

Amino acid analysis consists of two parts, hydrolysis of the protein to amino acids and the quantitation of the level of the amino acid in the hydrolysate. Standard methods generally use a chemical hydrolysis procedure; however, these methods tend to be expensive and time consuming, requiring strong acid or base solutions, high temperatures, and reaction conditions that are not suited to high-throughput analyses. Enzymatic hydrolyses are much more amenable to high-throughput procedures. Maize is problematic because the zeins are not soluble in the conditions under which most proteases function optimally. To alleviate this problem, we hydrolyze maize protein at pH 2, a condition that solubilizes maize proteins efficiently. We use the digestive enzyme pepsin, which functions well in these conditions.

Three types of methods are normally used to quantify target amino acids: bioassay, chemical assay, or chromatography. The American Organization of Analytical Chemists recognizes ion-exchange chromatographic methods for the determination of amino acids; however, the relatively low throughput and high cost of this method make it poorly suited to primary screening in plant-breeding programs. Chromatographic methods are well suited to verifying the results of more high-throughput, lower-cost methods because of their accuracy and acceptance by the scientific community.

Bioassays are low-cost high-throughput methods that are well suited to analysis in plant-breeding programs. Shankman et al. (1943) used strains of *Lactobacillus arabinosus* that are auxotrophic for specific amino acids to determine the concentrations of eight amino acids. The content of the amino acids was determined based on the amount of lactic acid produced by the bacteria. In 1995, Wright and Orman proposed another microbiological method for the analysis of methionine in maize and soybean seeds. They used the bacteria *Pediococcus cerevisiae,* which is auxotrophic for methionine, and measured the turbidity, a representation of bacterial growth, as an indication of the methionine content in the sample. Although this method may not provide the best analytical accuracy, it provides the high throughput required by plant breeders (Wright and Orman, 1995).

Hernandez and Bates (1969) determined that microbial assays and chromatographic techniques used in the determination of tryptophan were expensive, tedious, and time consuming. They developed a chemical method using iron chloride to characterize papain-hydrolyzed protein in terms of tryptophan content. This method was used extensively in the maize-breeding program at CIMMYT. In 1985, Sastry and Tummuru proposed a different method for analyzing the protein hydrolysates for tryptophan. After alkali hydrolysis of the sample, this method takes advantage of the colored product of the reaction between tryptophan, thioglycolic acid, and sucrose under acid conditions to measure tryptophan levels spectrophotometrically. This method is highly sensitive, rapid, and simple (Sastry and Tummuru, 1985).

## Divergent selection for tryptophan and methionine in two maize populations

### *Materials and methods*

#### Populations used in this study

Two different maize populations were used in this study. One population was derived from BS11, a population originally designated as Pioneer Two-Ear Composite. It was developed by crossing southern prolific material and Corn Belt lines (Hallauer, 1967). The second population was derived from BS31, another random-mated synthetic population derived from FS8A(T)C4 (Lamkey, 2002). The FS8A population was initially developed at the Florida Agricultural Experiment Stations and released in 1988. Germplasm from southeastern United States, Corn Belt, and tropical sources, respectively, account for approximately 30%, 22%, and 48% of FS8A(T). The initial development of this population consisted of intermating a wide range of accessions with resistance to southern corn leaf blight (Horner, 1990). The BS11 and BS31 material used in this study has been under selection for agronomic performance for several cycles of recurrent selection.

#### Breeding strategy

One hundred and two hundred half-sib ears from the populations BS11 and BS31, respectively, were produced in the summer of 2000 at the Iowa State University Agronomy Farm, analyzed, and categorized based on their methionine and tryptophan content. The five ears with the highest value of each amino acid and the five ears with the lowest value for each amino acid were selected from each popu-

lation, giving eight categories, each containing five selected ears. These categories were called BS11HT, BS11LT, BS11HM, BS11LM, BS31HT, BS31LT, BS31HM, and BS31LM. Thus, the BS11HT category represents the ears from the BS11 population with the highest content of tryptophan (HT), whereas BS31LM represents the ears from BS31 with the lowest content of methionine, and so on.

In the summer of 2002, a balanced bulk was made from each of the five ears selected in each category in 2000. Each of these eight bulks was planted in five adjacent rows with 25 kernels per row. The plants in each bulk were randomly intermated so that each plant that was used as a male was also used as a female, giving about 40 half-sib ears in each category. The resulting ears were harvested individually and the tryptophan and methionine content was analyzed as described below. Five selections from the approximately 40 ears in each category were chosen on the basis of their amino acid content as before. Taken together, these five selected ears constitute the cycle 1 population for each category.

#### Preparation of samples for analysis of methionine and tryptophan levels

Each ear of maize was shelled and packaged individually. From each ear, five randomly selected whole kernels were ground to a fine powder using a Wiley Mill with a 40-mesh screen. This powder was stored in Eppendorf tubes. With the flap of the tube open, the samples were then dried for four hours at 65°F, after which the tubes were closed and stored in ambient conditions. Samples were analyzed in 96-well plates using a Randomized Complete Block Design including two checks (B101 and B45o2) and six standards consisting of known concentrations of commercially prepared amino acids. The B101 inbred was chosen as a check for its exceptionally high levels of methionine (Hallauer and Wright, 1995). The B45o2 inbred, an *opaque-2* mutant, was used as a check for high tryptophan. The standard concentrations were 5, 20, 35, 60, 75, 100 μM for methionine and 0, 100, 240, 300, 480, 600 μM for tryptophan. The experiment was replicated on three plates (i.e., three blocks). The checks were replicated twice within a plate and the standards three times within a plate. Ten milligrams of each ground sample and checks were weighed into the well of a V-bottom, 96-well microtiter plate.

#### Protein hydrolysis

Each sample was subjected to enzymatic hydrolysis using pepsin. To each well, 200 μL of 0.2 mg/mL pepsin solution in a KCl-HCl pH 2 buffer was added. The plate was then sealed, covered with a lid, and placed in a 37°C shaking incubator for approximately 15 hours. After the incubation period, the plate was centrifuged at 3000 rpm for 20 minutes, after which the supernatant was removed for analysis.

#### Assay for tryptophan

The method for the determination of tryptophan in maize kernels is a modified version of the one originally described by Sastry and Tummuru (1985). Twenty microliters of hydrolysate or standard was transferred directly into the wells of a flat-bottom, 96-well assay plate. The plates were sealed between operations to prevent evaporation. For each plate, the assay solution consisted of 9.5 mL of concentrated HCl, 250 μL 2.5% thioglycolic acid, and 250 μL 10% sucrose. This solution was prepared and warmed to 42°C for 23 minutes to allow the solution to turn yellow. Eighty microliters of this assay solution was added to the hydrolysate in the assay plate. The plate was then shaken for three minutes, after which the optical density at 510 nm was immediately determined with a microplate reader.

#### Assay for methionine

The microbiological method for the determination of methionine in maize kernels is similar to that described by Wright and Orman (1995). An auxotrophic strain of *Escherichia coli*, P4x, was used in this assay. The inoculum was prepared in M9 media (Maniatis et al., 1982) supplemented with 10 μL of 1 mg/mL methionine solution per 5 mL of M9 media and grown to late log phase. Ten microliters of hydrolysate or a standard was transferred directly into a flat-bottom, 96-well assay plate. The plates were sealed between operations to prevent evaporation. To each well, 100 μL of M9 media and 2 μL of the inoculum were added. The plate was then sealed, covered with a lid, and placed in a 37°C shaking incubator for seven hours. After the incubation period, the plates were placed on a plate shaker for three minutes, and the 595 nm light scattered by the sample was determined using a microplate reader.

### Statistical analysis

In our amino acid assays, the greatest source of error is plate-to-plate variation between each replication of a sample. To correct for this, the mean value of the samples on each plate and the grand mean of the samples on all three plates in each experiment were calculated. Each value from a given plate was then normalized by multiplication by the value required to make the mean of that plate equal to the grand mean of the experiment.

## *Results*

### Tryptophan and methionine concentrations in starting populations

To determine the feasibility of direct selection for tryptophan and methionine content, populations derived from BS11 and BS31 that are under investigation for their agronomic traits were chosen on the basis of their protein content and their variability. One hundred and 200 individuals from the respective populations were analyzed for their tryptophan and methionine content. Figures 24.2

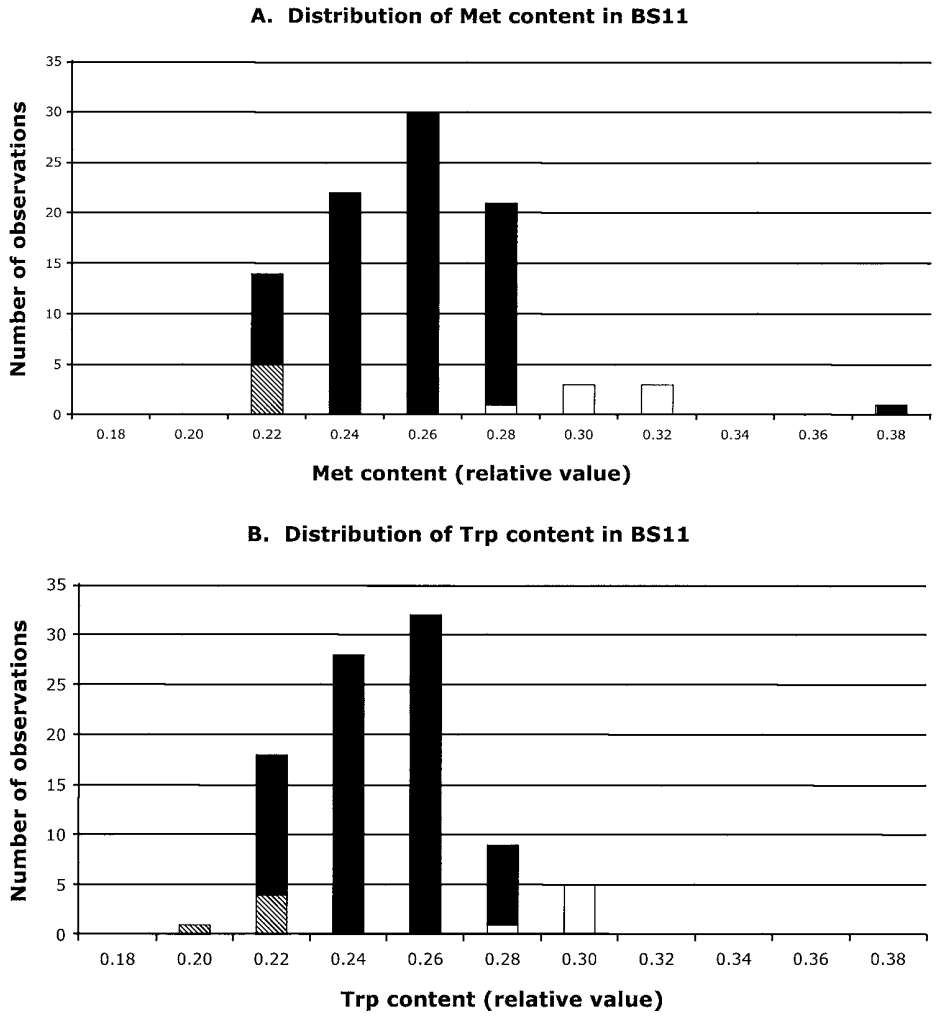


**Figure 24.2** Distribution of methionine content (a) and tryptophan content (b) in the initial BS11 population. The levels of methionine and tryptophan represent the optical density measurement corrected for the mass of the sample. The selections made for cycle 1 of the recurrent selection program for the low categories (BS11LM and BS11LT) are cross-hatched. The selections for the high categories (BS11HM and BS11HT) are in white. The overall population mean methionine content was 0.25 and the mean tryptophan content was 0.24.

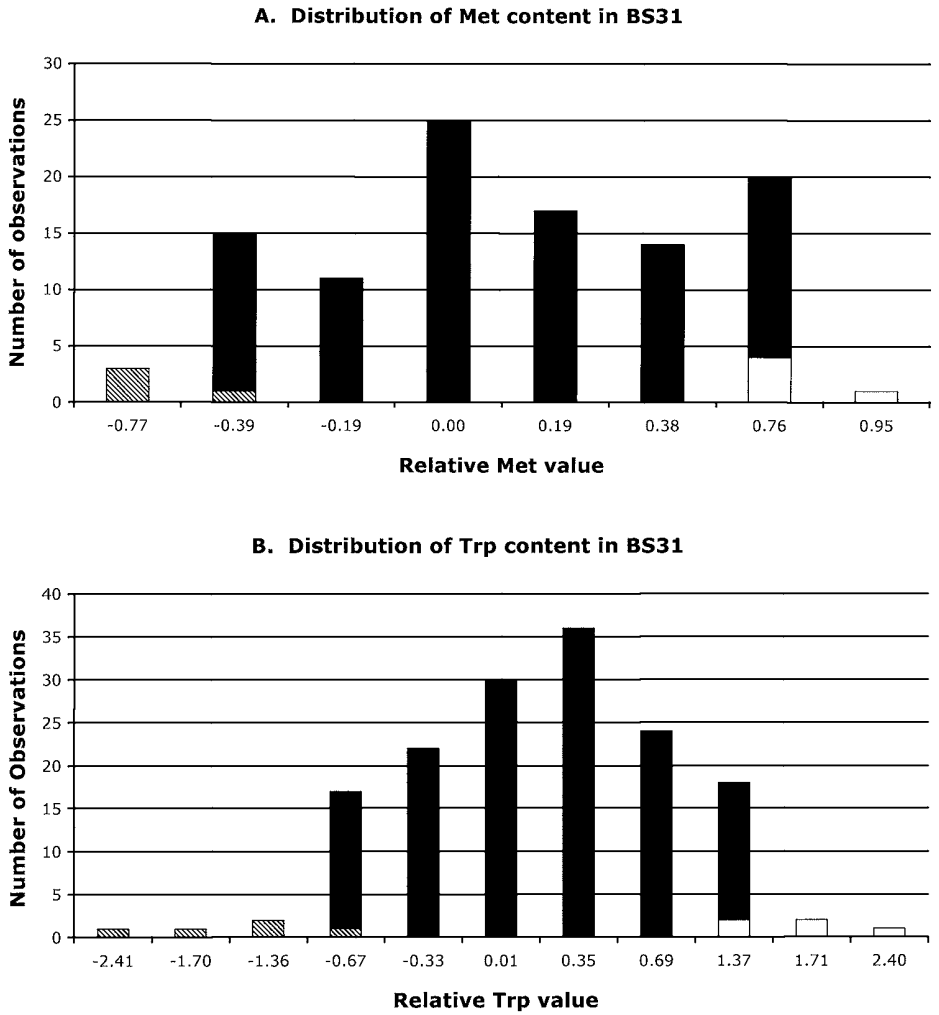


**Figure 24.3** Distribution of methionine content (a) and tryptophan content (b) in the initial BS31 population. The methionine and tryptophan values are relative to the overall mean value for the population. The selections made for cycle 1 of the recurrent selection program for the low categories (BS31LM and BS31LT) are crosshatched. The selections for the high categories (BS31HM and BS31HT) are in white.

and 24.3 show the distributions of tryptophan and methionine content in these individuals. Selections were made within these two populations to generate high and low subpopulations from each starting population. In the BS11 population, the mean tryptophan content of the selections in the high category was 28% higher than the mean of the selections in the low category. Similarly for the methionine content, the mean of the high category was 29% higher than the mean of the low selections. The distribution of the starting BS31 population is given relative to the mean of the population for each trait. There was more variation for tryptophan (relative values of ±2.40 from the mean) than for methionine (relative values of −0.77 for the low tail and +0.95 for the high tail). These selections formed eight new populations, four from BS11 and four from BS31, that were selected either for high or low tryptophan or methionine levels.

#### Effect of selection on random-mated populations

We completed one full cycle of selection by intermating among the selections within each population in the summer of 2002. This allowed us to evaluate the potential of recurrent selection for

**Table 24.1** Mean Met and Trp content for each category of the cycle 1 populations derived from BS11 and BS31 and their statistical analysis for mean comparisons of the categories for each trait in each experiment

A. Mean Met and Trp values

| | | Population[a] | |
|---|---|---|---|
| Trait | Category | BS11 | BS31 |
| Met | HM | 0.1643 | 0.1757 |
| | LM | 0.1533 | 0.1736 |
| Trp | HT | 0.3030 | 0.2985 |
| | LT | 0.2849 | 0.2916 |

B. Results of F tests for mean comparisons

| | Population | |
|---|---|---|
| Contrast | BS11 | BS31 |
| HM vs. LM | b | n.s. |
| HT vs. LT | b | n.s. |

*Note:* All statistically significant differences show that the mean value of the high category is higher than the mean value of the low category.

[a]Relative values for the trait represented by the optical density measurement corrected for the mass of the sample.

[b]significant at alpha = 0.05

n.s., not significant.

changing amino acid levels. Approximately 40 ears resulting from intermating among the selections within each population were analyzed for their tryptophan and methionine content. The mean tryptophan and methionine values for each category are reported in Table 24.1. Statistically significant differences in methionine and tryptophan levels were observed between the high and low populations derived from BS11. For both tryptophan and methionine levels, the high category was found significantly higher than the low category. The mean methionine content of BS11HM was 7.15% higher than the mean of BS11LM. Similarly, the mean of BS11HT was 6.37% higher than the mean of BS11LT. The tryptophan and methionine populations derived from BS31 did not have statistically significant differences in methionine and tryptophan levels. However, the mean tryptophan content of BS31HT was 2.39% higher than the mean of BS31LT, and the mean methionine content of BS31HM was 1.23% higher than the mean of BS31LM.

## *Discussion*

### Effect of selection

To investigate the effect of selection for tryptophan and methionine content in maize populations, we completed one cycle of divergent selection for tryptophan and methionine concentrations in two populations. The data suggest that a divergence in tryptophan and methionine levels is possible.

The response to selection for methionine and tryptophan was greater in the BS11-derived populations than in those derived from the BS31 populations. Several possibilities may explain the different responses to selection in these two populations. There may be greater genetic variability in BS11 than BS31. A second possible explanation could be that we are observing genetic drift because of our small population sizes (Keeratinijakal and Lamkey, 1993). These issues will be clarified upon completion of more cycles of selection.

These data illustrate the feasibility of direct selection for tryptophan and methionine in two maize populations. The differences in gain from selection in the two populations underscore the importance of identifying an appropriate population for this type of experiment. Upon completion of more cycles of selection, these populations will be valuable tools for the development of inbred lines with altered amino acid balance. They will also serve as a tool for studies of genetic factors controlling tryptophan and methionine levels in grain.

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